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Histological Changes At The Site Of Embryo Implantation In Albino Rats

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Abstract

Introduction: In many women, attachment and implantation doesn't happen and this is a major cause of miscarriage. By understanding how this process works, we may be able to inform the development of drugs to help embryos implant properly.
Aim of work: Studying the events of structure changes that could develop in the endometrium at the time of implantation.

Material and methods: thirty five ♂and seventy♀ female adult rats were used in the study; Each male rat was breeded with two female rats in a cage under hygienic condition. Female rats were devided into seven groups, ten rats for each. The first group was the control group. Other groups included rats at the second, the third, the fourth, the fifth, and the sixth days of pregnancy respectively. Daily vaginal smears were taken and the stage of estrous cycle was determined. After 1,2,3,4,5,and6 days of pregnancy female pregnant rats as well as female control rats were sacrificed .The uterus specimens were taken and prepared for paraffin sections ,stained with H & E, Mallory and PAS stains as well as immunohistochemical staining for Vimentin. Sections were subjected for the image analyzer system for measurement of the optical density of PAS and statistical analysis.

Results : Light microscopy examination of sections of the pregnant rat uterus revealed that at the first day there was decidualization (the cells become large and vaculated). At the second day , the uterine glands decreased in number and there was infiltration with inflammatory cells. At the third and fourth days , the uterine cell mass increased while the endometrial glands decreased in number. At the fifth day , the uterine cell mass increased more and more (deciduoma), the blastocyst appeared in the uterine cavity and implantation began at this stage. The wall of blastocyst was lined by trophoplastic cells. At the sixth day , the emberyonic cell mass surrounded by trophoplastic cells implanted in the decidua. Comparing with control uterus PAS reaction was concentrated in the endometrial cells and in the trophoplastic cells and around the emberyo. Vimentin stain was negative in the endometrial cells and around the emberyo in the sixth day comparing with the control.

Conclusion: Implantation is a complex process including proliferation of endometrial cells, decidualization, increase blood flow and immune cells enrichment. There is a difinit molecular harmony between the embryo and the endometrium, which allows them to interact and implantation to occur .A part of this may be Vimentin digestion which may be one of the prostaglandin functions.

Introduction

The successful implantation of the blastocyst depends on adequate interactions between the embryo and the uterus. The development of the embryo begins with the fertilized ovum, a single totipotent cell which undergoes mitosis and gives rise to the blastocyst. At the same time, increasing concentrations of ovarian steroid hormones initiate a complex signaling cascade that stimulates the differentiation of endometrial stromal cells to decidual cells, preparing the uterus to lodge the embryo. Studies in humans and in other mammals have shown that cytokines and growth factors are produced by the pre-implantation embryo and cells of the reproductive tract; however, the interactions between these factors that converge for successful implantation are not well understood. In many women, attachment and implantation don't happen and this is a major cause of miscarriage (PCASRM, 2006). By understanding how

this process works, we may be able to inform the development of drugs to help embryos implant properly. Implantation relies on a set of closely coordinated events occurring between a very early stage embryo and the lining of the uterus. The embryo must initially attach and form a contact with the lining of the uterus. (Kennedy, 2003). The embryo and the endometrium interact to each other, .In case of implantation, the trophoplastic cells ,establish contact with the prepared endometrium of the mother .Failure to iniciate the critical early events of implantation results in early pregnancy failure Castro-Rendón *et al.* (2006): In addition to processes occurring in the embryo during the peri-implantation period, uterine events also may be considered in a developmental context. The uterus undergoes dynamic changes during the cycle and displays many features including differential and ordered activation of gene expression and programmed changes in post transcriptional and post translation modification of mRNA and proteins. While the progression of these events is largely driven by endocrine actions, they display the same sequentional nature as classical developmental processes (Fazleabas and Strakova, 2002).In the presense of an emberyo, the endometrium is maintained, and progress through an additional program of events. Prostaglandins and PGE2 in particular, binds to its specific receptor (EP2 or EP4) and activates adenyl cyclase. The resulting increase in intracellular levels of cAMP can now activate IGFBP-1 gene transcription at the site of implantation. Epithelial responses and Stromal differentiation is initiated Fazleabas ,*et al.* (1999). However , decidualization requires a signal from the conceptus i.e., the dicedual cell response, leading to prolonged maintainance and additional programs of gene expression that are not observed (Kennedy,2003). Lymphoid wandering cells, mast cells and leukocytes are present in the endometrial stroma at all stages of cycle. In the early cycle ,the stromal cells are immature , as the cycle advances , they differentiate into fibroblast like cells and further maturation occurs in the later part of the cycle as they become decidual cells . The main function of the stroma is supportive through the production of collagen. Also, it services a nutritional function to the invading blastocyst after implantation (Weinke *et al.*, 1968).

Intermediate filaments (IFs) represent one of the prominent cytoskeletal elements of cells. Their constituent proteins are coded by a multigene family. They determine the shape of the nucleus and the cell more generally, their nanomechanical properties effect the stability and plasticity of cells and tissues Parry *et al.* (2007) Vimentin is one of the intermediate filaments present in the cytoplasm of cells of mesenchymal origin including endothelial cells , myofibroblasts ,some smooth muscle cells. has a direct relation to prostaglandins.In some cells it establishes a structural link between the plasma membrane and nuclear lamins. (Keita *et al.* , 2008).

Material And Methods

Adult rats were used, 35 males and 70 females, their age was between 4-6 months. Their weight was between 200-250 grams. Each male rat was breeded with two female rats in a cage at room temperature and under aseptic condition and were feeded by ordinary diet. The female rats were divided into seven groups each contained ten female rats, the First group was the control group, the Second group was at the age of one day of pregnancy with the age of 1 cell in oviduct ,the Third group was at the age of two days of pregnancy with the age of 4 cells in oviduct ,the Fourth group was at the age of three days of pregnancy with the age of 8-12 cells in oviduct , the Fifth group was at the age of four days of pregnancy with the age of morula at the end of oviduct ,the Sixth group was at the age of five days of pregnancy i.e the age of free blastocyst in uterus ,and the Seventh group was at the age of six days of pregnancy i.e the age of implanting blastocyst with trophoblastic cone and inner cell mass . Daily vaginal smears were taken and the stage of estrous cycle was determined. The next day, the presence of a vaginal plug or spermatozoa in the vaginal smear was designated as day 1 of pregnancy. Once the pregnant females were detected they were labeled by a card in wich the date of conception has been written. After 1, 2, 3, 4, 5 and 6 days of the

detected fertilization the female rats as well as control rats were taken and sacrificed under general anesthesia using ether. The abdomen is longitudinally dissected,the uterus was identified, dissected and cut transversely into small pieces and placed in neutral buffered formol saline . Specimens were processed and 6 μm serial paraffin sections were obtained.

Paraffin sections were prepared and stained with H&E, Mallory, PAS stains according to Drury and Wallington 1980 as well as immuno - histochemical staining for Vimentin according to Gustafsson *et al* ., 1988. Sections were subjected for the image analyzer system for measurement of optical density of PAS and subsequent statistical analysis.

Results

Examination of serial thin sections of the pregnant rat uterus with light microscopy revealed that at the first day , there was decidualization ie.the cells become large and vaculated (Fig., 4). At the second day , in addition to decidualization, the endometrial glands decreased in number and there was infiltration of inflammatory cells (Fig., 5). At the third day there was decidualization, increase uterine cell mass, while the endometrial glands showed a marked decrease in number. (Fig., 6). At the fourth day there was decidualization, increase in uterine cell mass, and decrease in the endometrial gland (Fig., 7).At the fifth day there was decidualization, increase in the uterine cell mass more and more (deciduoma), decrease endometrial glands, the blastocyst appeared in the uterine cavity and implantation began at this stage. The wall of blastocyst was lined by trophoplastic cells (Fig., 8 & 9). At the sixth day there was decidualization, (deciduoma), the endometrial glands became few in number, the emberyonic cell mass was surrounded by trophoplastic cells implanted in the decidua. (Fig., 10). PAS reaction showed positive reaction in the endometrium of control rats as well as the endometrial cells of pregnant rats from the first day to the sixth day and in the trophoplastic cells and around the emberyo. The peak of increase was higher at the second day pregnancy with MOD value (0.217353) and lower in the third day with MOD value (0.17151) respectively (Fig.,12,13,14 & 20-Table,1). Immune staining for Vimentin was negative in the endometrial cells and around the emberyo in pregnant rat at the fifth day and the sixth day comparing with the control rat and pregnant rats before implantation where endometrial cells showed a positive reaction. (Fig., 18 &19). Mallory stain showed increase in orange red red stained tissue in the endometrial cells from the first day to the sixth day and around the emberyo in the implantation site comparing with control uterus (Fig., 16 & 17).

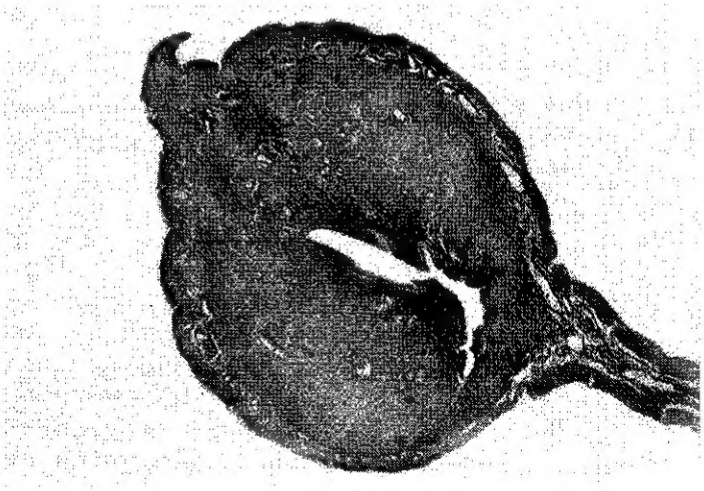

Fig. (1) Section of the uterus of a control rat showing thick endometrium with numerous endometrial glands. (H &E X50

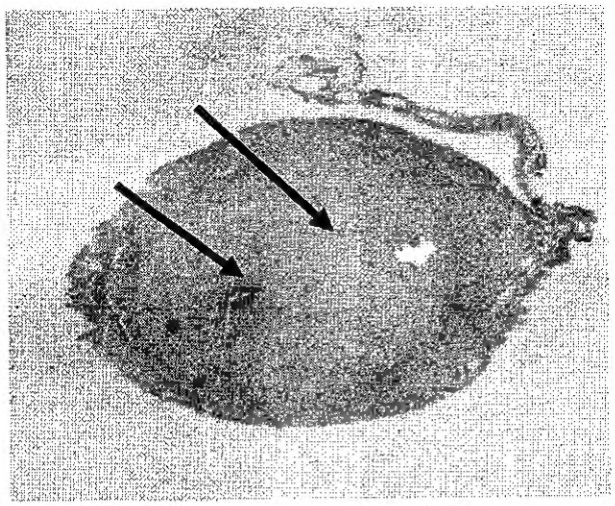

Fig.,(2) Section in the uterus of a pregnant rat at the sixth day showing deciduoma (black arrow) and the embryonic tissue (red arrow) , the endometrial cavity became very small. (H &E X50)

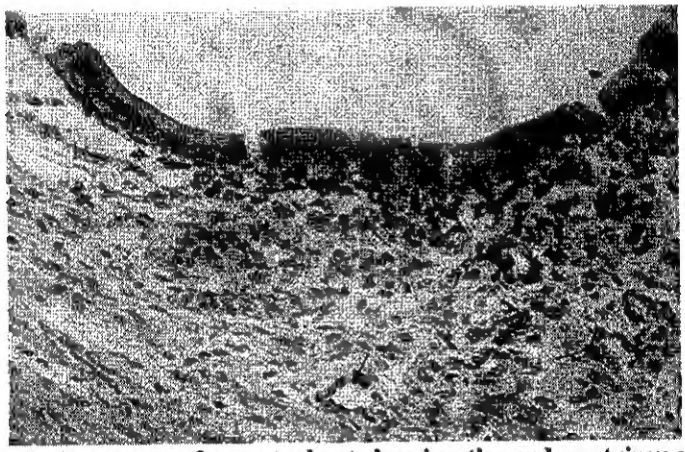

Fig.,(3) Section in the uterus of a control rat showing the endometrium with numerous endometrial glands (red arrows). (H &E X500)

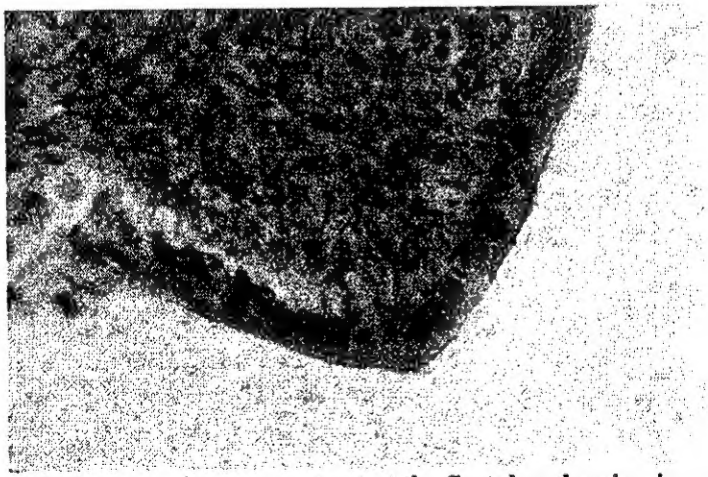

Fig.,(4) Section in the uterus of a pregnant rat at the first day showing increase in size and number of endometrial cell (decidualization) (H &E X500)

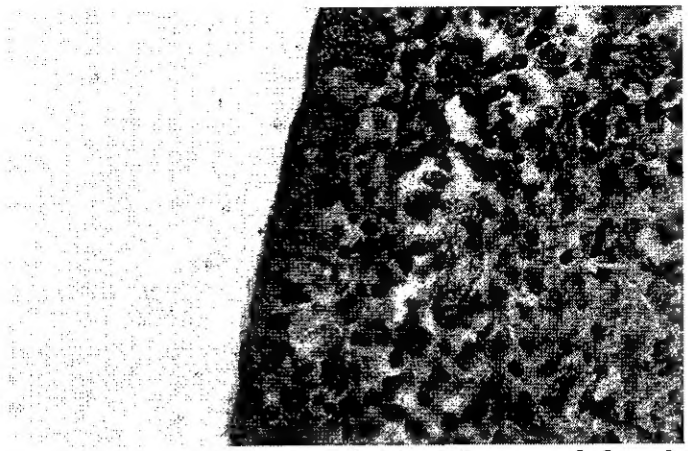

Fig. (5) Section in the uterus of a pregnant rat at the second day showing large and vacuolated endometrial cells ,decidualization (yellow arrow) and infiltration with inflammatory cells (red arrow) (H&E X50)

Fig. (6) Section in the uterus of a pregnant rat at the third day showing decidualization (yellow arrow) and infiltration of inflammatory cells(red arrow) . (H&E X500)

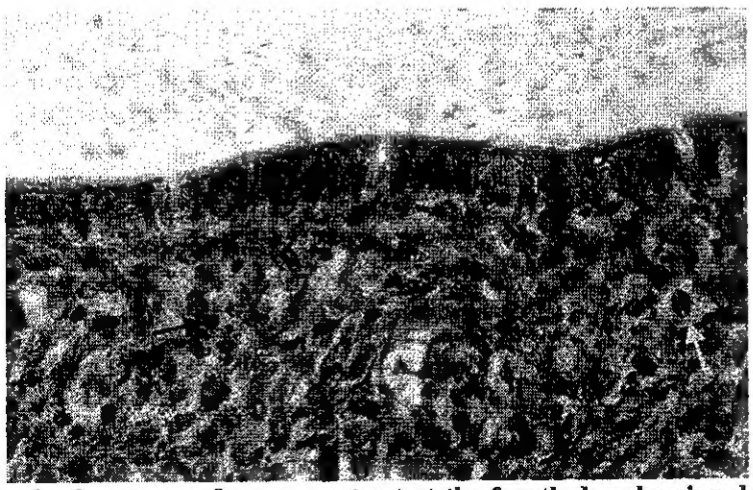

Fig. (7) Section in the uterus of a pregnant rat at the fourth day showing decidualization (yellow arrow) and infiltration of inflammatory cells (red arrow). (H&E X500)

Fig. (8) Section in the uterus of a pregnant rat at the fifth day shows the early implantation of the plastocyst with its trophoplastic cells (yellow arrow) and the endometrial gland (red arrow). (H&E X200)

Fig. (9) Section in the uterus of a pregnant rat at the fifth day showing the early implantation of the plastocyst with its trophoplastic cells (yellow arrow), the embryonic cleft (black arrow) and the endometrial gland (red arrow). (H&E X400)

Fig. (10) Section in the uterus of a pregnant rat at the sixth day showing emberyonic tissue surrounded by trophoplastic cells (Red arrow) and decidualization. (Yellow arrows). (H&E X500)

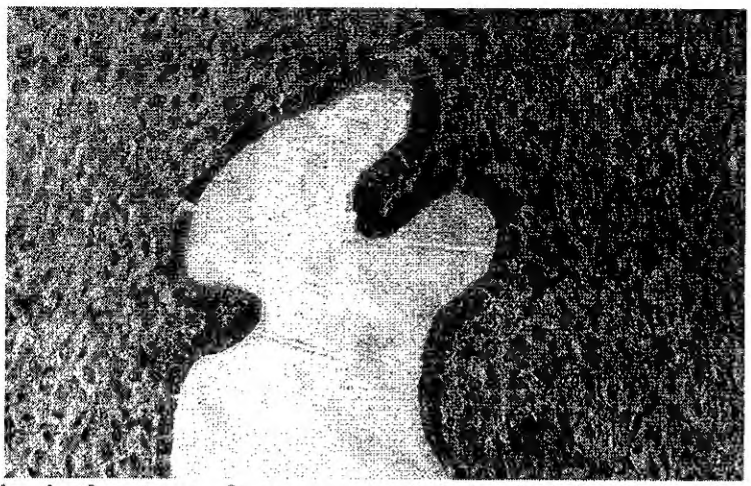

Fig. (11) Section in the uterus of a pregnant rat at the sixth day 6 showing regression of the endometrial glands . (H&E X500)

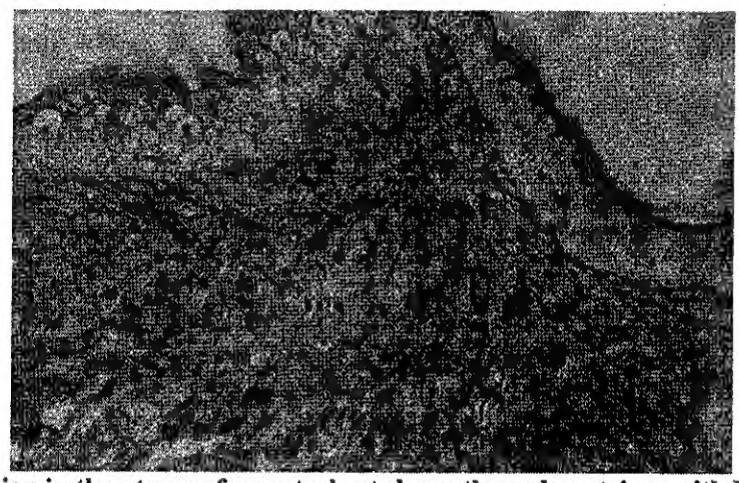

Fig.,(12) Section in the uterus of a control rat shows the endometrium with PAS reaction. (PAS X500)

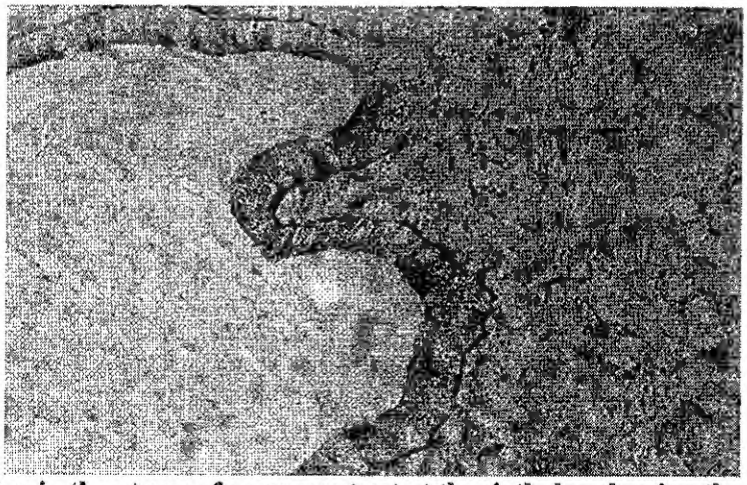

Fig. (13) Section in the uterus of a pregnant rat at the sixth day showing the endometrium with PAS positive material in endometrial epithelium. (PAS X500)

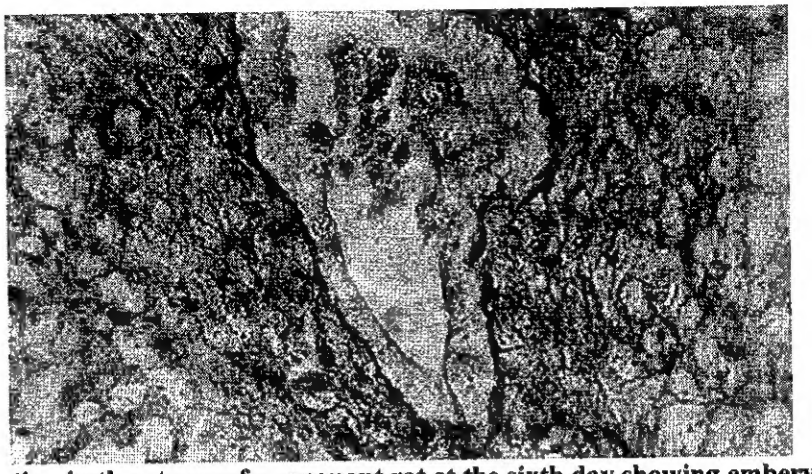

Fig. (14) Section in the uterus of a pregnant rat at the sixth day showing emberyonic tissue surrounded by trophoplastic cells with increase PAS positive material. (PAS X500)

Fig.,(15) Section in the uterus of a control rat showing the normal distribution of collagen (blue),nuclei and other connecting elements (red) . (Mallory X500)

Fig. (16) Section in the uterus of a pregnant rat at the fourth day 4 showing an increase in the ratio of red stained endometrial elements (connecting elements). (Mallory X500)

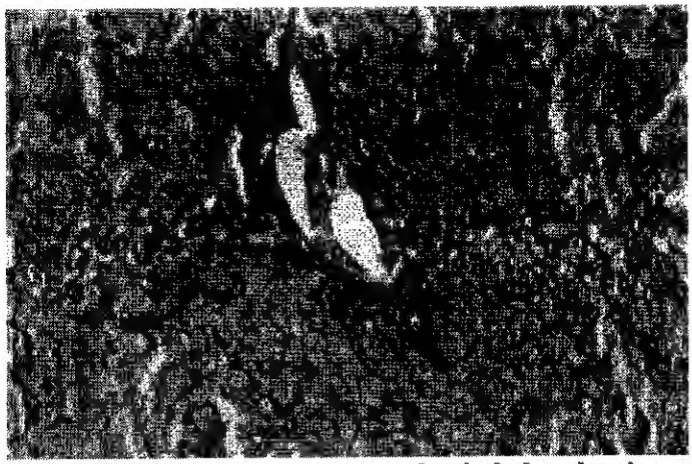

Fig. (17) Section in the uterus of a pregnant rat at the sixth day showing emberyonic tissue surrounded by trophoplastic cells with increase red colour (red arrow). (Mallory X500)

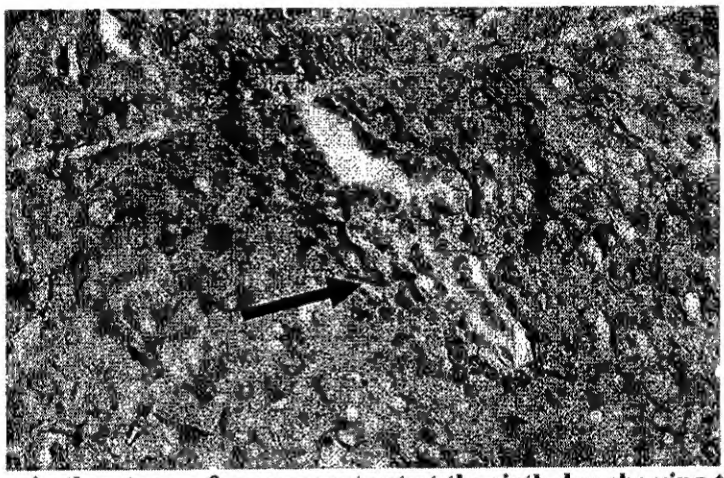

Fig. (18) Section in the uterus of a pregnant rat at the sixth day showing the emberyonic tissue surrounded by trophoplastic cells with negative immunohistochemistry for Vimentin indicated with violet colour (red arow). (Vimentin X500)

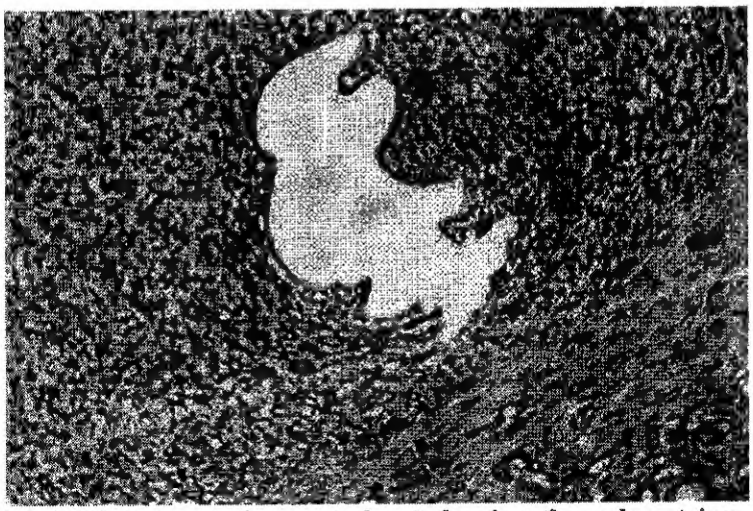

Fig. (19) Section in the uterus of a control rat showing the endometrium with positive immunohistochemistry for Vimentin (red arrow) (Vimentin X250)

Table (1): Mean Optical Density values (M.O.D.) of PAS positive material in the endometrium of different groups

GROUP	Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
M.O.D.	0.212584	0.187179	0.217353	0.17151	0.191732	0.2011	0.206731

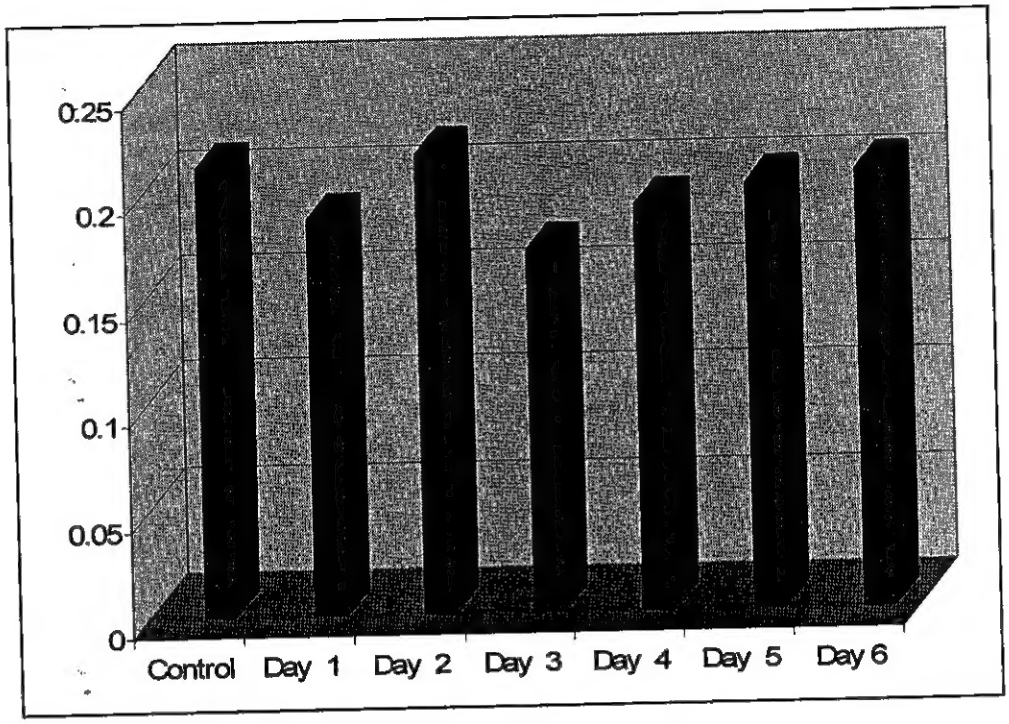

Fig. (20) Histogram representing the relation between the mean optical density values of PAS positive material in the endometrium of different groups.

Discussion

The endometrial luminal epithelium has an essential role in decidualization as indicated by the work of (Lejeune *et al.*, 1981), who demonstrated that if the luminal epithelium is destroyed decidualization cannot be obtained in response to stimuli (Mark *et al* ., 2007) , One of the earliest forms of embryo-maternal communication is the physical contact between the embryo and the epithelium for implantation. Therefore understanding what occurs during the process of implantation may give us a clue to determine some of the possible causes of failure of assisted reproductive teqniques and habitual abortion. It has been proposed that as the microvilli of the trophoplast interdigitate with those of the epithelium, pulsation of the plastocyst during this period might lead to distortion of the epithelium and augment a physical signal (Fishel and Surani, 1978 and Pollheimer and Knofler, 2005). In the present study implantation in the albino rats occurs at the sixth day of pregnancy . Detection of pregnancy at albino rats depended on determination of different phases of the estrus cycle and detection of mucous blug to determine the exact day of pregnancy. In the present work the endometrium showed decidualization and infiltration with inflammatory cells. Inflammatory cells secrete cytokines

associated with implantation and their exact role aren't understood completely (Kimber, 2005). Also ,we found that the number of endometrial glands was decreased in the pregnant uterus than in the control group ,this was in agreement with the study of Rudolf and Melven, (1986) and Mark et al., (2006), as the role of endometrial glands nutrition may be carried by the future placenta (Cavagna and Mantese 2003). As regards to PAS reaction, it was concentrated in the endometrium and around the emberyo ,and this increase could be explained by the increase in glycogoprotein secretion by both endometrial epithelium and decidual cells and reflects the interaction between the embryonic and decidual cells . Protein and glycoproteins have been detected from cells of the blastocyst of numerous animal species. It has been clear that a number of protein and glycoprotein secreted into the luminal fluid during the process of implantation is stimulated by changing ovarian steroid ratio (Fishel and Surani ,1978 and Familiari et al., 2008). The distribution of Mallory stain in the pregnant endometrium reflects the presence of collagen in the deep layer of endometrium while the red colour of orange G noticed in the superficial zone of endometrium and around the embryo suggested a decrease in collagen fibers, this supports the results of the immune stain an could help implantation. Similar results were reported by (Clark *et al.*,1993 and Tominaga , 1996). The expression of intermediate filaments (Ifs) is essential for successful decidualization and implantation as Vimentin plays a role in cell growth and mitosis (Korgun *et al.*, 2007). Vimentin was used to study the relation between the intermediate filaments and implantation. Immunohistochemical staining for Vimentin was positive in the endometrial cells of both controle and early five days pregnancy and this may explain the role of Vimentin as an intermediate filament in the endometrial decidualization and growth. However Vimentin stain was negative at the sixth day of pregnancy in the endometrial epithelium and in decidual cells around the embryo, Ikeda *et al.* (2008) reported that most of the blastocysts did not exhibit immunostaining for the vimentin protein at implantation, this result suggests that inhibition of vimentin is essential for implantation Vimentin inhibition may help the plastocyst in the process of penetration of the decidua and this role might be played by prostaglandins specially PGE2 (Simmons *et al.*, 2004). This suggestion is supported by the role played by Vimentin in tumour cell behaviour as Vimentin increases mitosis and spread of tumour cells and inhibition of Vimentin protein suppresses mitosis and spread of these cells (Zhao, *et al.*, 2008 and Zhonghua *et al.*, 2008). The implantation of the blastocyst into the endometrium involves the initial unstable adhesion of the blastocyst to the endometrial surface called apposition followed by a stable adhesion phase and decidualization of the endometrial stroma. Trophoblast – mediated attachment and subsequent implantation depend on the uterine luminal epithelial cell membrane – bound and soluble form of heparin - bound epidermal growth factor, a member of the transforming growth factor – ά family and strong binding affinity of the epidermal growth factor for a specific receptor (Dey *et al.*, 2004). At implantation cytoplasmic processes of trophoblastic cells interact with small processes on the apical surface of the uterine epithelial cells called pinopods and penetrate the intercellular spaces of the endometrial luminal cells. Penetration is facilitated by a decrease in the number of desmosomes linking the endometrial cells that undergo apoptosis The primary decidual zone is remodeled by the action of PGs and a secondary decidual zone houses the implanted embryo (Abraham, 2006). PGs have an important role in the early events of implantation and artificially induced decidualization. However, specificity of PGE2 remains controversial. There may be differences between species, and different PGs may be involved at different times, (Tomas *et al.*, 2007).

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التغيرات التركيبية في مكان اندماج الجنين فى الجرذان البيضاء

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عند اندماج الجنين فى الرحم فان خلايا التروفوبلاست المحيطة بالجنين تنشئ اتصالا خاصا مع جدار الرحم فى فترة حرجة واذا لم يتم خلال هذه الفترة اندماج الجنين فى الرحم عادة ما يحدث اخفاق مبكر للحمل . وبالإضافة الى كثير من الأحداث التى تحدث للجنين قبل واثناء مرحلة الاندماج فان هناك تغيرات رحمية معتبرة تحدث فى سياق تطورى حيث يتحمل الرحم تغيرات فعالة اثناء الدورة ويعرض له تغيرات عدة منها افراز بروتينات مختلفة والتى تؤثر فى عملية الاندماج الرحمى . وتقود الهرمونات تقدم هذه الأحداث بشكل واسع كما ان النسيج الطلائى المبطن لتجويف الرحم يلعب دورا اساسيا فى عملية اندماج الجنين , وقد ثبت ذلك فى دراسات سابقة منشورة بالدوريات العلمية .وقد هدفت هذه الدراسة الى محاولة فهم ما يحدث اثناء عملية اندماج الجنين فى الرحم مما قد يعطينا دلالة لفهم اسباب إخفاق الإخصاب المساعد والإجهاض المتكرر .لذا صممت هذه الدراسة على سبعين من اناث الفئران البيضاء البالغة حيث قسمت الى مجموعات بعد التاكد من حدوث الحمل لهن جميعا وذلك باختبار المخاط المهبلى يوميا و قد تم اخذ عينات من جدار الرحم من المجموعات يوميا على فترة ستة ايام بعد ذبحهن لاختبار التغيرات الهستولوجية التى قد تحدث فى جدار الرحم يوميا ثم تم عمل شرائح من البارافين وتم صباغتها بصبغات الهيماتوكسيلين والايوسين ومالورى الثلاثية وكذلك بتفاعل الباص كما تم صبغ بعض الشرائح بمادة الفايمنتين الهستوكيميائية المناعية وبفحص النتائج تبين ان خلايا الرحم تزداد فى الحجم وتتسلل بعض خلايا الالتهاب الى الرحم ويزداد تدفق الدم فى الاوعية الدموية التى تغذى النسيج الرحمى ويزداد تركيز الجليكوجين فى النسيج الطلائى المبطن للرحم وخاصة فى الخلايا المحيطة بالجنين وهذا يعنى ان النسيج الطلائى المبطن للرحم وكذلك خلايا الجنين كلاهما مسئول عن عملية الاندماج الجنينى , وقد لاحظنا ان تركيز صبغة الفايمنتين ضعيف جدا فى النسيج الطلائى المبطن للرحم حول الجنين مما قد يعنى ان عدم وجود الفايمنتين قد يساعد الحوصلة الجنينية على اختراق النسيج الطلائى المبطن لتجويف الرحم وكذلك خلايا الرحم , وتلعب البروستاجلاندينات دورا فعالا فى ذلك , ويدعم هذا البحث فرضية اشتراك كلا من الجنين وجدار الرحم فى عملية اندماج الجنين.